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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

No statistical method was used to predetermine sample size. Sample size was predetermined according to statistical data analysis of previous studies utilizing similar models of hematopoiesis done in our laboratory. See citations in the Statistics parts of the Methods section in the manuscript.

2. Data exclusions

Describe any data exclusions.

Sample exclusion was done only as a result of premature mouse death or infection due injury or improper healing post surgery.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts of replication were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice were randomly assigned to experimental groups. All experiments were repeated in both male and female mice. For all experiments comparing mice at different ages, age and sex matched mice were randomly assigned to each test group.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

No sample blinding was utilized for either data collection or analysis. Data blinding was not possible for most experiments due to obvious difference in mouse size and weight between young and old mice. Denervation experiments compared samples derived from the same mouse (denervated versus sham limb)

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Data analysis and presentation was performed using either GraphPad Prism 7 (GraphPad Software, San Diego, CA), FACS Diva 6.1 software (BD Biosciences), FlowJo 10.0.8 (LLC), Slide Book software (Intelligent Imaging Innovations) and Fiji build of ImageJ (NIH). All statistical analyses were performed using GraphPad Prism 7.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All unique materials used in this study are available upon request. No restrictions apply.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The following antibodies were used for flow cytometry: anti-Ly6A/E-Alexa Fluor 700 (D7; 56-5981-82), anti-CD117-PE/Cy7 (2B8; 105814), anti-CD45.2-PE (104; 109807), CD45- Pacific Blue (30-F11; 103126), anti-CD31-Alexa Fluor 647 (MEC13.3; 102516), anti-VE-cadherin (CD144)-APC-Alexa Fluor 647 (BV13; 138006), Anti-CD150-PE (TC15-12F12.2; 115904), anti-Ter119-Pacific Blue (TER-119; 116232), anti-CD16/32 (FcγR III/II)-APC/Cy7 (93; 101328) were purchased from BioLegend (San Diego, CA). Anti-CD48-PerCp-eFluor710 (HM48-1; 46-0481-182), anti-CD45.1-FITC (A20; 11-0453-85), anti-CD4-PE/Cy7 (GK1.5; 25-0041-82), anti-CD8a-PE/Cy7 (53-6.7; 25-0081-85), anti-B220-APC-eFluor 780 (RA3-6B2; 47-0452-82), anti-CD11b (Mac-1)-APC-eFluor 780 (M1/70; 47-0112-82), anti-Gr-1 (Ly-6G)- APC-eFluor 780 (RB6-8C5; 47-5931-82), anti-CD3e-APC-eFluor 780 (145-2C11; 47-0031-82), anti-CD45-APC-eFluor 780 (30-F11; 47-0451-82), anti-CD31-PE/Cy7 (MEC13.3; 25-0311-82), anti-PDGFRα (CD140a)-APC (APA5; 17-1401-81), anti-PDGFRα (CD140a)-PE/Cy7 (APA5; 25-1401-82), CD51-PE (RMV-7; 13-0512-85), anti-CD41-FITC (MWReg30; 11-0411-81), anti-Ki-67-PerCp-eFluor 710 (SolA15; 46-5698-80), anti-Ter119-APC-eFluor 780 (TER-119; 47-5921-82), Streptavidin APC-eFluor 780 (47-4317-82), anti-CD34-eFluor 660 (RAM34; 50-0341-82 at 1:50 dilution), anti-CD127 (IL7R)-PerCp (A7R34; 45-1271-80), CD135 (Flt3)-PE (A2F10; 12-1351-83) were purchased from eBioscience (Thermo Fisher). Anti-lineage panel cocktail (TER-119, RB6-8C5, RA3-6B2, M1/70, 145-2C11 at 1:50 dilution) was from BD Biosciences (559971). Unless otherwise specified, all antibodies were used at a 1:100 dilution.
Antibodies used for immunofluorescence imaging: Anti-lineage panel cocktail (TER-119, RB6-8C5, RA3-6B2, M1/70, 145-2C11 at 1:50 dilution) from BD Biosciences (559971), anti-CD48-biotin (HM48-1; 13-0481-80, 1:100 dilution), CD41-biotin (MWReg30; 11-0411-82, 1:2500 dilution) from eBioscience (Thermo Fisher) and anti-CD31-Alexa Fluor 647 (MEC13.3; 102516), anti-CD144 (VE-cadherin)-APC-Alexa Fluor 647 (BV13; 138006), Anti-CD150-PE (TC15-12F12.2; 115904 at 1:100 dilution) from Biolegend (San Diego, CA). Anti-Tyrosine Hydroxylase (TH) antibody (Cat: AB152; Lot: 2493925; Millipore), Alexa Fluor 488 conjugated anti-Tubulin Beta3 (Tubb3) antibody (Clone: AA10; Cat: 657404; Biolegend), rabbit polyclonal anti-Synaptophysin (Cat: ab32594; Abcam), rabbit polyclonal anti-Perilipin antibody (Clone: D1D8; Cat: 9349; Cell Signaling Technology), monoclonal anti-Actin α-Smooth Muscle - Cy3-conjugated antibody (Clone: 1A4; Cat: C6198; Sigma), Alexa Fluor 568-conjugated goat anti-rabbit IgG (Cat: A11011, Invitrogen), FITC-conjugated anti-phospho H2A.X (ser139) antibody (Clone: 2F3; Cat: 613404; Biolegend), rabbit polyclonal anti-Cdc42 antibody (1:50 dilution, Cat: 07-1466; Millipore), rat monoclonal anti-Tubulin antibody (1:100 dilution, Clone: YL1/2; Cat: ab6160; Abcam), Alexa Fluor 633-conjugated goat anti-rabbit IgG (Cat: A21071; Invitrogen) and Alexa Fluor 488-conjugated goat anti-rat IgG (Cat: A11006; Invitrogen).

All flow cytometry antibodies were anti-mouse and their specificity was validated in previous studies performed in our laboratory.
Most imaging antibodies were validated in previous studies done by our laboratory, the specificity of other antibodies was validated by comparing antibody staining with staining of isotope control antibodies and secondary antibody only staining.
Please citations in the Online Methods part of the manuscript for our previous studies using similar antibodies.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Eukaryotic cell lines were not used in the study.

b. Describe the method of cell line authentication used.

N/A

c. Report whether the cell lines were tested for mycoplasma contamination.

N/A

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

N/A

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Nestin–GFP mice were bred and aged in our facilities. Aged C57BL/6 mice were obtained from the National Institute of Aging (NIA), or retired breeders from Jackson Laboratory, or by aging mice in our facilities for 8, 12 and 24 months. Young C57BL/6 mice were purchased from the National Cancer Institute or the Jackson laboratory (C57Bl/6J). Adrb3−/− mice were from the Jackson Laboratory (FVB/N-Adrb3tm1Lowl/J; stock: 006402) and back-crossed to C57BL/6 background in our facilities. JaB6-Ly5.1 (CD45.1) mice were purchased from the National Cancer Institute or the Jackson laboratory (B6.SJL-Ptprca Pepcb/BoyJ). Unless indicated otherwise, 8 to 10-week-old male and female mice were used as young controls and 20 to 24-month-old males and females were designated as old mice. For all analytical and therapeutic experiments, sex-matched animals from the same age group were randomly assigned to experimental groups.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Nucleated single-cell suspensions enriched from bone marrow were obtained by flushing and dissociating BM plugs using a 21G needle in phosphate-buffered saline (PBS; Gibco). Single-cell suspensions enriched from peripheral blood were obtained by retro orbital or cheek bleeding of mice. For analysis of stromal and endothelial cell populations, intact flushed bone marrow plugs were digested for 30 min at 37°C in 1 mg ml-1 Collagenase type IV (Gibco) and 2 mg ml-1 Dispase (Gibco) in Hank's balanced salt solution (HBSS; Gibco). Compact bones (after flushing out marrow) were crushed and digested in 3 mg ml-1 Collagenase type 1A (Sigma) in 1% FBS/HSBSS for 90 min rotating at 37°C. For FACS analysis or sorting, cells were stained with antibodies in PEB (PBS containing 0.5% BSA and 2 mM EDTA). Dead cells and debris were excluded by FSC, SSC and DAPI (4', 6-diamino-2-phenylindole; Sigma) staining profiles. To eliminate the non-endothelial CD31+ fraction, total ECs quantified by FACS were identified as CD45− Ter119− CD31high Sca-1+ and arteriolar ECs were identified as CD45− Ter119− CD31high Sca-1high. For sorting ECs, anti-CD31-Alexa Fluor 647 (MEC13.3; 102516) was injected intravenously 10 min prior to sacrificing mice.
6. Identify the instrument used for data collection.	BD LSRII Special Order System (BD Bioscience) was used for all data acquisition (H55100027). BD FACSAria IIu (P46900051) and BD FACSAria II (P69500137) Special Order Systems were used for most sorting experiments. MoFlo Astrios EQ (Beckman coulter) was used for sorting HSCs for RNA-seq analysis and imaging.
7. Describe the software used to collect and analyze the flow cytometry data.	Data was collected using BD FACSDiva 6.1 (BD Biosciences) software Data was analyzed with FACSDiva 6.1 and FlowJo V.10.1 (LLC) softwares
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Purity of cells sorted or analyzed was determined by their appropriate frequency and absolute numbers determined for control wild-type young mice according to previous studies. It was described in the literature that HSCs comprise around 0.01% of total bone marrow. We have previously shown that MSCs and ECs are 0.05% and 0.05-0.1% respectively, of total bone marrow. In every experiment, we included a control group comprised of a cohort of young control mice. Because the populations we were sorting are very rare, we did not do post

sort analysis.

9. Describe the gating strategy used.

For all flow cytometric analysis and sorting debris were excluded by FSC, SSC scatters and DAPI (4', 6-diamino-2-phenylindole) staining was used to exclude dead cells, following which specific population were gated according to prior experience with doing similar experiments in our laboratory . Briefly, for mesenchymal stem cell (MSC) gating: CD45+ Ter119+ CD31+ cells were excluded by staining with CD45, Ter119 APC-Cy7 or Pacific Blue and CD31-PE-Cy7. MSCs were identified, as previously described, to be CD51-PE+ and CD140a (PDGFRa)-APC positive within the CD45- Ter119- CD31- triple negative population. Gates were determined by using specific fluorescence minus one (FMO) controls.
Endothelial cells (ECs) were identified as CD45- Ter119- and positive for CD31-PE-Cy7 and Sca-1 - Alexa Fluor 700. To eliminate the non-endothelial CD31+ fraction, total ECs quantified by FACS were identified as CD45– Ter119– CD31high Sca-1+ and arteriolar ECs were identified as CD45– Ter119– CD31high Sca-1high.
Detailed gating strategies for stromal cell populations and cell cycle analysis are presented in Fig. 1f, Fig. 3d, Fig. 3e, Fig. 4h, Supplementary Fig. 1a, Supplementary Fig. 7a and Supplementary Fig.8f.
Hematopoietic stem cells (HSCs) were identified as previously described in the literature: negative for lineage (CD3e, B220, Gr-1, CD11b, Ter119)-APC-Cy7, negative for CD48-PerCp and positive for Sca-1-Alexa fluor 700, CD117 (c-Kit)-PE-Cy7 and CD150-PE (SLAM markers)
All other FACS analyses - Ki67 positivity, donor derived CD45.2 cells in transplantation setting, myeloid cells (CD11b+), B cells (B220+), T cells (CD4+/CD8+) have all been described previously (see manuscript for citations describing similar studies done in our laboratory)

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒